

can be introduced into a DNA to be labeled through nucleotide-adding reaction with terminal transferase. This is one of requirements for the nucleotide compounds to be utilized in the 3'-tailing labeling method. Such nucleotides that meet the above-mentioned requirement include inosinic acid and xanthylic acid. Among them, inosinic acid is a readily available and a preferred nucleotide in the functional aspect, achieving the effect of the present invention. For example, a nucleotide sequence containing deoxyinosinic acid can be added to the 3' end of a DNA by incubating it with terminal transferase in the presence of deoxyinosine 5'-triphosphate as a substrate.

It is generally believed that inosinic acid can contribute to base pairing with some affinity irrespective of the type of partner base. Because of this, it has been thought that the introduction of inosinic acid to label a sequence, as in the present invention, does not reduce but enhance nonspecific reaction. Actually, however, it is possible to greatly reduce the nonspecific reaction by introducing inosinic acid in a sequence to be added.

The hybridization probe based on the present invention or the method for providing the probe, which essentially contains nucleotides exhibiting weak base pairing, also contains other nucleotides (e.g., a, t, c, g, and u, or derivatives thereof). These nucleotides may be labeled. However, the hybridization should be carried out under conditions where the portion added for labeling cannot hybridize to any sequences when the probe contains nucleotide components other than the above-mentioned nucleotides and/or nucleotide derivatives exhibiting weak base pairing. Preferred conditions are conditions where the nucleotide sequence to be added *per se* cannot hybridize to any nucleotide sequences under stringent hybridization conditions for the DNA to be labeled.

In order to achieve such conditions, it is necessary to increase the proportion of the above-mentioned nucleotides (or nucleotide derivatives) exhibiting weak base pairing in the sequence to be added for labeling. Since the minimum proportion depends on the type of nucleotide, the composition of co-existing nucleotides, and the total length, it is difficult to show the typical range. However, those skilled in the art can empirically determine the proportion based

on the disclosure of the present invention. Any of the nucleotides or nucleotide derivatives added to the DNA to be labeled can be subjected to the labeling. Accordingly, labeled inosinic acid can be used in combination with non-labeled nucleotides. In this case, the spacer can consist of non-labeled nucleotides alone, or alternatively the spacer can also contain non-labeled inosinic acid together with the labeled one. For example, when the labeling by the tailing is carried out with deoxyinosine 5'-triphosphate (a nucleotide with weak affinity) and digoxigenin-labeled deoxyuracil 5'-triphosphate, the latter is used in a proportion of 10% or lower and thereby the conditions of the present invention are successfully achieved.

The terminal transferase to be used in the 3'-tailing labeling method adds nucleotides at random to the 3' end of a DNA without distinguishing the type of base. Therefore, the proportion of bases in the nucleotide sequence to be added should be adjusted based on the types of nucleotides used as substrates and the concentration ratio thereof. A specific example in respect to the proportion is exemplified as follows: when deoxyinosine 5'-triphosphate is used in combination with digoxigenin-labeled deoxyuracil 5'-triphosphate label, the concentration of the former is adjusted to 2 to 10 times excess over that of the latter to expect the improvement of specific activity of the label and the inhibitory effect on the nonspecific reaction. Since labeled nucleotides and derivatives thereof generally tend to be less efficient as substrates in the addition reaction with terminal transferase, an adequate concentration ratio should be determined empirically based on the type of label and the types of nucleotides used in combination. It is impossible to strictly control the nucleotide sequence based on the concentration ratio alone. However, based on the disclosure of the present invention, those skilled in the art can readily determine conditions under which accidentally hybridizable nucleotide repeats is not formed.

On the other hand, it is preferable to use, for example, a label sequence consisting of deoxyinosinic acid alone when it is necessary to increase the composition ratio of nucleotide exhibiting weak base

pairing. Such an embodiment can be achieved by combining labeled deoxyinosinic acid as a labeled nucleotide. When the type of base used is deoxyinosinic acid alone, labeling by 3'-tailing can be performed under conditions where a labeled probe with the highest specific activity can be provided at the optimal concentration ratio between labeled deoxyinosine 5'-triphosphate and non-labeled deoxyinosine 5'-triphosphate.

The 3'-tailing labeling method is already known (Molecular Cloning, Cold spring harbor laboratory press, 1989). Specifically, terminal transferase is allowed to act on DNA (or oligonucleotide) to be labeled in the presence of labeled nucleotide and spacer (non-labeled nucleotide) as substrates. In the present invention, a nucleotide such as deoxyinosine 5'-triphosphate may be added as the spacer nucleotide for the reaction. While the terminal transferase commonly used is derived from calf thymus, there is no particular limitation on the origin. The reaction solution can contain a buffer agent for the optimal pH of the reaction, an agent for maintaining enzymatic activity such as bovine serum albumin, or salts such as cobalt chloride, which provide metal ions required for the expression of the enzyme activity. Terminal transferase (1 to 10 units) is added to the solution and the mixture is incubated at 37°C for about 15 minutes to achieve the addition of nucleotide sequence for labeling.

It is preferable to unite into single reaction conditions to minimize variations of specific activity between the batches. For this purpose, stopping agents for the reaction can be used to control reaction time. For example, when glycogen and ethylenediaminetetraacetic acid (EDTA) are added to the reaction, the enzyme activity of terminal transferase is rapidly dropped and thereby the reaction is terminated.

In the present invention, labeled nucleotides or nucleotide derivatives, as well as nucleotides (or nucleotide derivatives) in the nucleotide sequence to be used for labeling may be any types of nucleotides. Even if the nucleotides for labeling scattered in the nucleotide sequence for labeling are nucleotides capable of forming usual base pairs, they are hardly hybridizable because of the random